REMARKS

By the foregoing amendments, claims 2 and 5 have been amended and claims 9-12 have been added. Support for the amendments to claim 2 can be found in the substitute specification at page 41, lines 13-22, and in Example 13, page 79, lines 13-17. The amendment to claim 5 is for consistency in terminology with claim 2. Support for new claims 9-12 can be found in the substitute specification at page 12, lines 8-11; page 41, lines 13-22; page 49, line 15 to page 52, line 13; and Example 2.

Also by the foregoing amendments, the specification has been amended at page 43, lines 10-15. Support for the amendment can be found in the amended paragraph, wherein it is stated that some of the SELEX targets may be proteins that recognize sites on RNA but do not engage in further catalytic action. Such recognition sites would be binding sites, as is known to those skilled in the art.

The Applicants acknowledge with thanks the withdrawal of the Section 112, second paragraph rejections.

Application Status and Effective Date

The Examiner maintains that pending claims 2 and 4-8 are entitled to an effective filing date of the amendment in which they were presented, i.e., February 13, 2002, because the subject matter is allegedly not supported by the disclosure. The Examiner's contention that the claims are not supported by the disclosure appears to be limited to lack of written description. Applicants respectfully traverse this finding.

The Applicants submit that the effective filing date of the pending claims is at least as early as the filing date of USSN 07/714,131, which is identical to the subject specification and to which the subject application claims priority. A detailed explanation of how the subject specification supports and fully describes the pending claims is set forth below.

The Rejection Under 35 USC § 112, First Paragraph

Claims 2 and 4-8 stand rejected under Section 112, first paragraph, because they allegedly contain subject matter which was not described in the specification in such a

way as to reasonably convey to the skilled artisan that the inventors had possession of the claimed invention at the filing date. Applicants respectfully traverse this rejection.

Claim 2 is directed to a method of identifying the binding site on a region of RNA or DNA to which a binding protein binds, using a nucleic acid ligand to the binding protein, by performing a competitive assay between the RNA/DNA region and nucleic acid ligand for binding to the binding protein. If the ligand inhibits binding to the RNA/DNA region, the ligand's sequence or other structure can provide information about the specific nucleotides or structure of the RNA/DNA region involved in binding to the binding protein.

A protein that simply binds RNA/DNA, and which does not have a separate active (i.e., catalytic/enzymatic) site, can effect through its binding some regulatory function on the polynucleotide (e.g., repressor, activator). Alternately, the binding of a binding protein which has a separate active site can, through assembly of necessary substrates at the binding locale, engage in replication or degradation reactions. For example, polymerases, replicases and reverse transcriptases bind to their binding sites on cognate RNA or DNA regions and, upon assembly of necessary substrates, engage in replication of polynucleotides along the template. Regardless of whether the binding protein has an active site separate from the binding site, the nucleic acid ligand to the binding protein can be useful in identifying the binding site on the RNA/DNA if the nucleic acid ligand binds to the binding site of the binding protein that binds to the binding site of the RNA/DNA. The binding of a nucleic acid ligand to the binding protein in a manner that blocks binding of the protein to the RNA/DNA can be assayed by determination of function of the binding protein: for example, does the ligand reduce the binding protein's repressor/activator function, or its replication or degradation function?

The following passages are reproduced from the specification as evidence of literal written support for claim 2 in its present form. At page 41, lines 13-22 of the substitute specification, it is stated:

> The method of the present invention has multiple applications. The method can be employed, for example, to assist in the identification and characterization of any protein binding site for DNA or RNA. Such binding sites function in transcriptional or translational regulation of gene expression, for example as binding sites for transcriptional activators or repressors, transcription complexes at promoter sites, replication accessory proteins and DNA polymerases at or near origins of replication and ribosomes and translational represssors at ribosome binding sites. Sequence information

of such binding sites can be used to isolate and identify regulatory regions bypassing more labor-intensive methods of characterization of such regions. Isolated DNA regulatory regions can be employed, for example, in heterologous constructs to selectively alter gene expression.

At page 47, lines 7 to 9 of the substitute specification, it is stated:

The methods of the present invention are useful for obtaining nucleic acids which will inhibit function of a target protein, and are particularly useful for obtaining nucleic acids which inhibit the function of proteins whose function involves binding to nucleic acid....

In Example 3, RNA ligands to bacteriophage R17 coat protein were identified. R17 coat protein represses R17 replicase RNA (Carey et al. (1983) Biochem. 22:2601). It was found that the "winning RNA motif", i.e., the ligand with the highest affinity for the coat protein, bore a direct relationship to the coat binding site on the natural R17 genome identified earlier though site-directed mutagenesis and binding studies (page 62, lines 28-29 of the substitute specification). Thus, in this example, *a nucleic acid ligand to a binding protein that has no catalytic activity and acts only as a repressor, is found to be directly related to the wild type binding site found in the natural genome.* This provides direct written description for subject claim 1.

In Example 13, nucleic acid ligands to HIV-1 rev protein are identified. The rev protein binds to the rev response element (RRE) in an HIV-1 mRNA. The RRE is reported to assume a complex configuration in its recognition of rev, and the rev-RRE interaction is believed to mediate export of HIV structural messenger RNAs from the nucleus to the cytoplasm (Olsen et al. (1990) Science 247:845). The SELEX method yielded 53 isolates which were analyzed in a secondary structure prediction program, along with the wild type RRE. It was found that nucleic acid ligands segregated into 3 different structural motifs. The folded sequences of representatives of each motif are shown in Figure 23 along with the folded sequence of the wild-type RRE. One domain of the wild-type RRE closely resembled Motif II. Thus, the structure of nucleic acid ligands can be and was used to elucidate the structure of the naturally-occurring mRNA that binds to the rev protein. Specifically, on page 79, lines 13-17 of the substitute specification, it is reported that the conservation of certain nucleotides and structures is common to Motifs I and II as well as to wild-type RRE. When a base pair substitution occurred in conserved nucleotides, the resulting ligand had a reduced affinity compared to most other Motif I sequences. This information provided identification of the binding site structure of RRE critical

for binding to the rev protein.

The Examiner also refers to Example 2, in which nucleic acid ligands are obtained to HIV-1 RT. The highest affinity ligand obtained is 1.1, which was found to significantly inhibit reverse transcriptase activity even at equal concentrations of 1.1 to RT. In contrast, starting population RNA significantly inhibited RT only at a 200-fold excess. This strong inhibitory effect of 1.1 was reported to indicate that the ligand "either blocks or directly interacts with the catalytic site of the enzyme" (page 61, lines 14-20 of the substitute specification).

The experiment of Example 2 does not appear to be *directly* concerned with elucidating the sequence or other structure of the binding site on the RNA to which the RT binds. Thus, it is not clear whether the ligand 1.1 blocked the catalytic site only, or if it also blocked the RT site that binds to the mRNA or tRNA. What is clear is that the relatively low concentration of 1.1 required to substantially inhibit activity of RT means that it must have been at least binding to or interacting with the catalytic site of RT.

The Examiner refers to page 66, lines 15-34, of the original specification (page 49, line 15 to page 52, line 13 of the substitute specification) in which the bacteriophage T4 DNA polymerase, gp43, is described. The gp43 protein is a binding protein that binds to its RNA at the operator to autogenously regulate its own production. The gp43 protein has a binding site for its cognate RNA transcript and an active site for its polymerase activity. One assay used to determine whether binding of gp43 to its transcript has occurred is the detection of polymerase activity.

The subject claim 2 is directed to a method of using a nucleic acid ligand to determine the binding site of a binding protein on the DNA or RNA. When the nucleic acid ligand binds to the binding protein and inhibits binding to its cognate RNA/DNA, the nucleic acid ligand, through its sequence or other structure, assists in the identification of the cognate RNA/DNA.

In the case of gp43, the nucleic acid ligand binds to the gp43 and inhibits binding of the gp43 to its cognate RNA. As a result of this binding inhibition, it also inhibits polymerase activity by gp43, not because it has bound to the active site but because it has bound to the gp43 binding site, causing the gp43 polymerase to become incapable of getting positioned on the operator. At page 66, lines 31-34 of the original specification (or page 50, lines 5-6 of the substitute specification), the specification states:

The addition of micromolar amounts of purified RNAs containing intact operator was

found to strongly inhibit in vitro replication by gp43.

At page 69, lines 30-33 of the original specification (or page 52, lines 11-13 of the substitute specification), the specification states:

> The loop sequence variant RNAs isolated by the selection/amplification process [i.e., SELEX], shown in Figure 7, can all act as inhibitors of gp43 polymerase activity as has been demonstrated for the wild-type operator sequence.

Thus, inhibition of gp43 binding to its cognate RNA by nucleic acid ligands of the subject invention, also has the effect of inhibiting the polymerase activity of gp43. Regardless of this secondary effect on polymerase activity, the nucleic acid ligand is still useful in the identification of the nucleotide sequence on the RNA/DNA that is involved in binding the gp43 binding protein.

Applicants have added new claims 9-10 to further clarify the nature of the invention. Claim 9 depends from claim 2 and specifies that the binding protein is an RNA or DNA replication enzyme and the determination of whether the nucleic acid ligand inhibits binding of the binding protein to the DNA or RNA region, is carried out by assaying for replication activity of the enzyme. It is possible, of course, that the nucleic acid ligand binds both the binding protein's binding site and active site. In this case, the nucleic acid ligand should still give information about the binding site on the RNA/DNA. It is also possible that the nucleic acid ligand binds only the binding protein's active site, in which case, it is unlikely to provide information about the binding site sequence or structure on the RNA/DNA. However, claims 2 and 9 are method claims, and claim 2 contains the functional phrase "if the nucleic acid ligand is inhibitory" of binding to the RNA/DNA. This effectively excludes any embodiments where the nucleic acid ligand is not inhibitory of binding of the binding protein to the RNA/DNA.

Claim 10 depends from claim 2 and provides that the determination of whether the nucleic acid ligand inhibits binding of the binding protein to the DNA or RNA region is carried out by assaying for repressor or activator activity of the binding protein.

Although Applicants believe claims 2 and 4-8 are patentable in their present form, the Examiner has requested Applicants to submit claims that have been "rewritten according to disclosed methods" (page 4, lines 20-21 of the Office Action). As an alternative, Applicants have added new claims 11 and 12 which are directed to a method of inhibiting the enzymatic activity

of a protein that binds to DNA or RNA, wherein the nucleic acid ligand may inhibit the binding of the protein to the DNA or RNA, and/or may bind to and inhibit the active site of the protein. These claims are offered as possibly being more acceptable to the Examiner. Also submitted herewith is a copy of U.S. Pat. 6,331,398, which is the parent of the subject application and which may be considered to have somewhat similar claims.

In view of the foregoing amendments and remarks, it is respectfully requested that the Section 112, first paragraph rejection be withdrawn.

The Rejection under 35 USC §§ 102 and 103

Claims 2, 3 and 5-8 stand rejected under Section 102(b) as anticipated by Giordano et al., U.S. 5,859,227. As is discussed above, the Applicants submit that the effective filing date of the subject claims is June 10, 1991. Therefore, the '227 patent is not considered prior art.

Claims 2, 3, 5, 6 and 8 stand rejected under Section 102(b) as anticipated by Weissman et al., U.S. 5,861,246. Claim 4 stands rejected under 102(b) as anticipated by, or in the alternative, under Section 103 (a) as obvious over the '246 patent. Because the effective filing date of the subject claims is June 10, 1991, the '246 patent is not prior art.

Withdrawal of the Section 102(b) and 103(a) rejections is respectfully requested.

Closing Remarks

It is believed that the foregoing amendments and remarks bring the subject case into condition for allowance and notification of same is respectfully requested. If the Examiner believes that a phone conference would expedite prosecution, she is invited to phone the undersigned.

Submitted herewith is a Petition for Extension of Time for 2 months and a check for $420.00. It is believed that no other fees are due with this submission. If this is in error, please

charge any necessary fees to Deposit Account No. 19-5117.

Respectfully submitted,

Date: June 16, 2004

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